

Structural insight into selectivity of amylin and calcitonin receptor agonists

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Peptide Synthesis and Characterization

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Supplementary table 1. Interactions between the peptides and receptors.

peptide	AMY ₃ R: San385	AMY ₃ R: San45	CTR: San45
K1	V293 ^{ECL2} , E294 ^{ECL2} (H bond)	V293 ^{ECL2} , E294 ^{ECL2} (H bond), Y299 ^{5.37}	E294 ^{ECL2} (H bond), V293 ^{ECL2} , Y299 ^{5.37}
C2	Y299 ^{5.37} , V293 ^{ECL2} (H bond)	V293 ^{ECL2} , Y299 ^{5.37} (H bond)	Y299 ^{5.37} , V293 ^{ECL2} (H bond)
N3	Y299 ^{5.37} , P360 ^{6.57} , W361 ^{6.58} , R362 ^{ECL3} (H bond), Y372 ^{7.38} (water mediated), P363 ^{ECL3} (water mediated)	P360 ^{6.57} , W361 ^{6.58} , R362 ^{ECL3} (H bond), Y372 ^{7.38} (water mediated)	Y299 ^{5.37} , R362 ^{ECL3} (H bond), W361 ^{6.58} , Y372 ^{7.38} (water mediated)
T4	P360 ^{6.57} , Y299 ^{5.37}	P360 ^{6.57} , Y299 ^{5.37} , M306 ^{5.44}	P360 ^{6.57} , W361 ^{6.58}
A5	P360 ^{6.57} , Y372 ^{7.38} , I380 ^{7.46} , F359 ^{6.56} , M376 ^{7.42}	Y372 ^{7.38} , I380 ^{7.46} , F356 ^{6.53} , F359 ^{6.56} , M376 ^{7.42} P360 ^{6.57} (H bond)	P360 ^{6.57} , Y372 ^{7.38} , I380 ^{7.46} , F356 ^{6.53} , F359 ^{6.56} , M376 ^{7.42}
T6	H302 ^{5.40} (H bond), V305 ^{5.43} , F356 ^{6.53} , Y234 ^{3.44} (water mediated)	Y234 ^{3.44} , H302 ^{5.40} (H bond), V305 ^{5.43} , F356 ^{6.53}	Y234 ^{3.44} , H302 ^{5.40} (H bond), F356 ^{6.53} , Y234 ^{3.44} (water mediated)
C7	H302 ^{5.40}	H302 ^{5.40}	H302 ^{5.40}
A8	H377 ^{7.43} (H bond), I380 ^{7.46}	H377 ^{7.43} (water mediated), I380 ^{7.46}	H377 ^{7.43}
T9	H381 ^{7.47}	H381 ^{7.47}	H381 ^{7.47} (water mediated), H156 ^{1.47} (water mediated)
Q10	L291 ^{ECL2} (water mediated), V293 ^{ECL2} , H226 ^{3.36} , Q227 ^{3.37} (H bond), M230 ^{3.40}	L291 ^{ECL2} (water mediated), V293 ^{ECL2} , H226 ^{3.36}	V293 ^{ECL2} , H226 ^{3.36} , H302 ^{5.40}
R11	D373 ^{7.39} (water mediated), Y372 ^{7.38} (water mediated)	D373 ^{7.39} (water mediated), Y372 ^{7.38} (water mediated)	D373 ^{7.39} (water mediated), Y372 ^{7.38} (water mediated), H377 ^{7.43} (water mediated)
L12	A145 ^{1.36} , L148 ^{1.39} , Y149 ^{1.40} , H377 ^{7.43}	A145 ^{1.36} , L148 ^{1.39}	A145 ^{1.36} , L148 ^{1.39} , Y149 ^{1.40}
A13	V206 ^{ECL1}	H201 ^{2.67}	/
N14	L291 ^{ECL2} (H bond), S292 ^{ECL2} (water mediated), V293 ^{ECL2} (water mediated), E294 ^{ECL2} (water mediated)	L291 ^{ECL2} , S292 ^{ECL2} (water mediated), V293 ^{ECL2} (water mediated)	L291 ^{ECL2} , S292 ^{ECL2} , V293 ^{ECL2}
F15	K141 ^{1.32} , A145 ^{1.36} , L142 ^{1.33}	D103 ^{ECD} , A145 ^{1.36} , K141 ^{1.32} , L142 ^{1.33}	F137 ^{1.28} , A145 ^{1.36} , A136 ^{1.27}
L16	V206 ^{ECL1} , Y149 ^{1.40} , A145 ^{1.36}	V206 ^{ECL1} , Y149 ^{1.40} , A145 ^{1.36}	V206 ^{ECL1} , Y149 ^{1.40} , Y146 ^{1.37}
V17	V206 ^{ECL1} , L291 ^{ECL2}	L291 ^{ECL2} , V206 ^{ECL1}	V212 ^{ECL1} , L291 ^{ECL2}
H18	/	P104 ^{ECD}	F137 ^{1.28} , P100 ^{ECD} , L40 ^{ECD}
K19^{San385} S19^{San45}	P100 ^{ECD} , L142 ^{1.33} , F102 ^{ECD} (H bond), D97 ^{ECD} (H bond)	L142 ^{1.33}	F137 ^{1.28} , L142 ^{1.33} , Y146 ^{1.37}
S20	Y146 ^{1.37}	/	/
N21^{San385}	/	P207 ^{ECL1} , V206 ^{ECL1} , Y149 ^{1.40} ,	P207 ^{ECL1} , Y146 ^{1.37} , Y149 ^{1.40} ,

K21 _{[Lira]^S an45}		V203 ^{2.69} , S157 ^{1.48} , I160 ^{1.51} , F161 ^{1.52} , Y150 ^{1.41}	I153 ^{1.44} , S157 ^{1.48} , Y150 ^{1.41}
N22	G209 ^{ECL1} , R213 ^{ECL1}	L142 ^{1.33}	P207 ^{ECL1} (H bond)
F23	P207 ^{ECL1} , Y146 ^{1.37} , Y149 ^{1.40}	Y146 ^{1.37} , L142 ^{1.33}	R213 ^{ECL1} (H bond), E210 ^{ECL1} , N208 ^{ECL1}
G24	/	/	R213 ^{ECL1} (H bond)
P25	/	/	R213 ^{ECL1} (H bond)
I26	/	/	R213 ^{ECL1} , G209 ^{ECL1} , V212 ^{ECL1}
L27	/	/	L40 ^{ECD} , P38 ^{ECD}
P28	/	/	/
P29	D101 ^{ECD}	D101 ^{ECD} , N135 ^{1.26}	D101 ^{ECD}
T30	D101 ^{ECD} (H bond), N135 ^{1.26} (H bond), F99 ^{ECD}	D101 ^{ECD} (H bond), N135 ^{1.26} (H bond), F99 ^{ECD}	D101 ^{ECD} (H bond), F99 ^{ECD} , W79 ^{ECD} , F102 ^{ECD}
N31	W79 ^{ECD}	W79 ^{ECD}	W79 ^{ECD}
V32	W128 ^{ECD} , Y131 ^{ECD} , N135 ^{1.26}	W128 ^{ECD} , Y131 ^{ECD} , F102 ^{ECD} , N135 ^{1.26}	W128 ^{ECD} , Y131 ^{ECD} , F102 ^{ECD} ,
G33	W128 ^{ECD} (H bond)	W128 ^{ECD} (H bond)	W128 ^{ECD} (H bond)
S34	N124 ^{ECD} , W128 ^{ECD} , H121 ^{ECD} , E123 ^{ECD} (H bond)	N124 ^{ECD} (H bond), R126 ^{ECD} , H121 ^{ECD} (H bond), E123 ^{ECD} (H bond)	W128 ^{ECD} , R126 ^{ECD} (H bond), H121 ^{ECD} , E123 ^{ECD} , N124 ^{ECD}
N35	N124 ^{ECD} , R126 ^{ECD}	R126 ^{ECD} (H bond)	R126 ^{ECD}
T36	R126 ^{ECD} , W128 ^{ECD}	R126 ^{ECD} , W128 ^{ECD}	R126 ^{ECD} (H bond), W128 ^{ECD}
Y37	R126 ^{ECD} , D77 ^{ECD} , W79 ^{ECD} , S129 ^{ECD} , W128 ^{ECD} , W84 ^{RAMP3} , E74 ^{RAMP3} (H bond)	W128 ^{ECD} , S129 ^{ECD} (H bond), G78 ^{ECD} , R126 ^{ECD} , W79 ^{ECD} , W84 ^{RAMP3} , E74 ^{RAMP3} (H bond)	Y131 ^{ECD} , S129 ^{ECD} (H bond), W128 ^{ECD} , G78 ^{ECD} , W79 ^{ECD}

Superscript numbers for CTR transmembrane residues use the conserved class B1 GPCR numbering system of *Wootten et al.*¹. ECD, CTR extracellular domain; ECL, CTR extracellular loop; RAMP3, interaction with residue from RAMP3.

Supplementary table 2. Cryo-EM data collection, refinement and validation statistics

	#1 AMY ₃ R:San385 (EMDB-28759, 28810 (Cluster 5)) (PDB 8F0K, 8F2A (Cluster 5))	#2 AMY ₃ R:San45 (EMDB-28812) (PDB 8F2B)	#3 CTR: San45 (EMDB-28758) (PDB 8F0J)
Data collection and processing			
Magnification	130k ×	130k ×	130k ×
Voltage (kV)	300	300	300
Electron exposure (e ⁻ /Å ²)	70.1	70.1	40.1
Defocus range (μm)	-0.5~1.5	-0.2~2.0	-0.2~1.5
Pixel size (Å)	0.65 (rescaled to 0.81)	0.65	0.65
Symmetry imposed	C1	C1	C1
Initial collected movies (no.)	6021	5867	6552
Final particle images (no.)	592k (Cluster 5: 118k)	518k	636k
Map resolution (Å)	1.9 (2.4 ^a) (Cluster 5:2.2, 2.4 ^b , 3.1 ^a)	2.0 (2.1 ^b)	2.0 (2.1 ^b)
FSC threshold	0.143	0.143	0.143
Refinement			
Initial model used (PDB code)	7TZF	7TZF	6NIY
Model resolution (Å)	1.94 (Cluster 5: 2.44)	2.09	2.13
FSC threshold	0.5	0.5	0.5
Map sharpening <i>B</i> factor (Å ²)	-35	-38	-44
Model composition			
Non-hydrogen atoms	10553 (Cluster 5: 10044)	10360	9678
Protein residues	1272 (Cluster 5: 1276)	1272	1156
Ligands	Y01:2, NAG: 5, PTY:1, PLM:8, P42:1 (Cluster 5: NAG:4)	Y01:2, NAG: 3, PLM:8, P42:1	Y01:2, NAG: 2, PTY:3, PLM:8, P42:1
<i>B</i> factors (Å²)			
Protein	29.7 (Cluster 5: 67.0)	13.32	10.71
Ligand	37.4 (Cluster 5: 183.5)	32.32	30.13
Water	30.0	19.22	18.17
R.m.s. deviations			
Bond lengths (Å)	0.003 (Cluster 5:0.003)	0.003	0.003
Bond angles (°)	0.520 (Cluster 5:0.580)	0.492	0.526
Validation			
MolProbity score	1.19 (Cluster 5:1.24)	1.30	1.20
Clashscore	4.05 (Cluster 5:4.68)	4.60	4.20
Poor rotamers (%)	1.00 (Cluster 5:0.76)	1.23	0.41
Ramachandran plot			
Favored (%)	99.04 (Cluster 5:98.33)	99.04	99.03

Allowed (%)	0.96 (Cluster 5:1.67)	0.96	0.91
Disallowed (%)	0 (Cluster 5:0)	0	0

¹Defocus range estimated from RELION

^a Resolution (Å) of ECD-focused cryo-EM map

^b Resolution (Å) of receptor-focused cryo-EM map

Supplementary Note

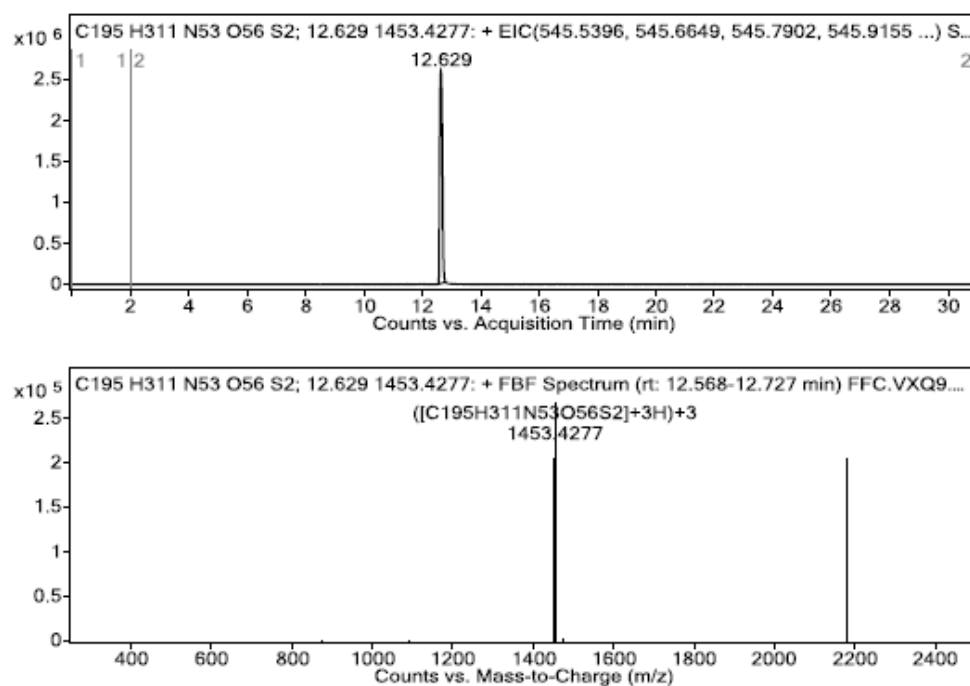
Supplementary Video Descriptions

Supplementary Video 1: 3DVA of cryo-EM data of San385-AMY₃R and San45-AMY₃R G protein complexes, shown sequentially, illustrating similar conformational dynamics of the RAMP3 c-terminus and ICL2 of the CTR protomer; the hydrophobic F256_{ICL2} is labelled. The map surface is coloured for individual protein components; red, RAMP3 (San385 complex), blue, CTR (San385 complex), orange, RAMP3 (San45 complex), light blue, CTR (San45 complex), gold, Gα_s ras domain, cyan, Gβ1, purple, Gγ2.

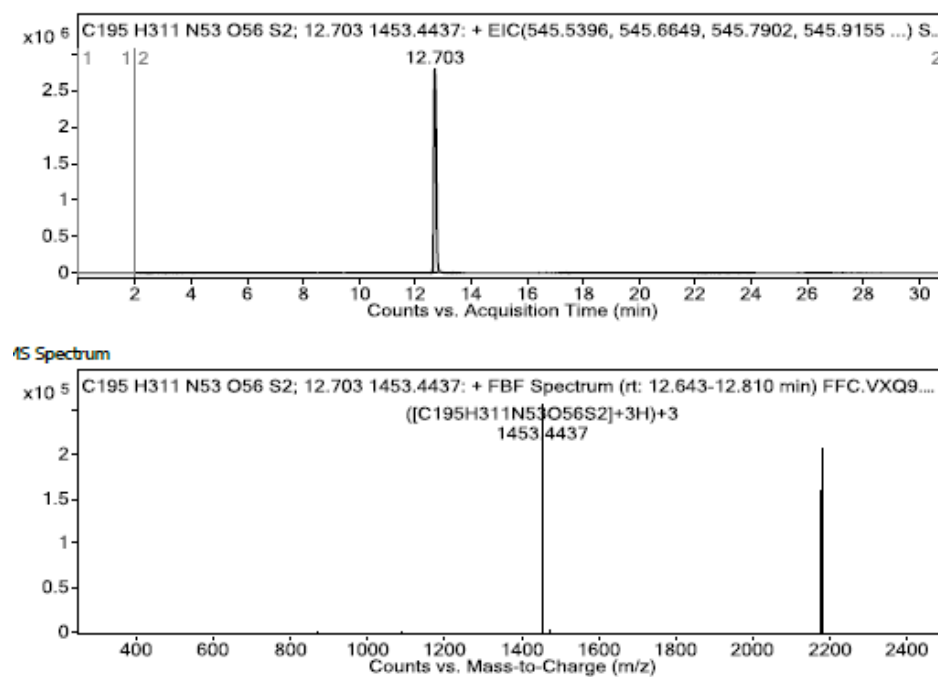
Supplementary Video 2: 3DVA of the first principal component of motion illustrating dynamics of the receptor ECD and peptides for the San385-AMY₃R complex. The map surface has been coloured for individual protein components; red, RAMP3, green, San385 and blue, CTR.

Peptide Synthesis and Characterization

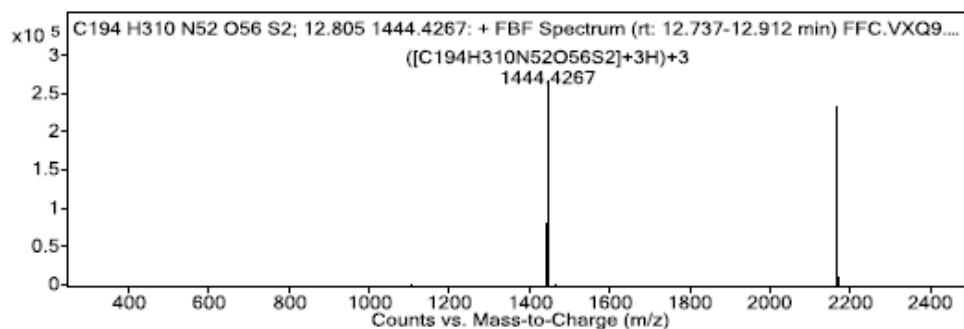
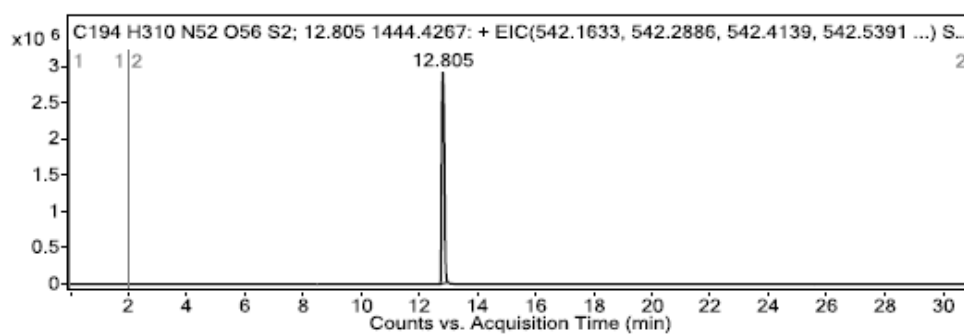
Analytical data, HPLC (top) and LC-MS (bottom), of purified pramlintide analogues



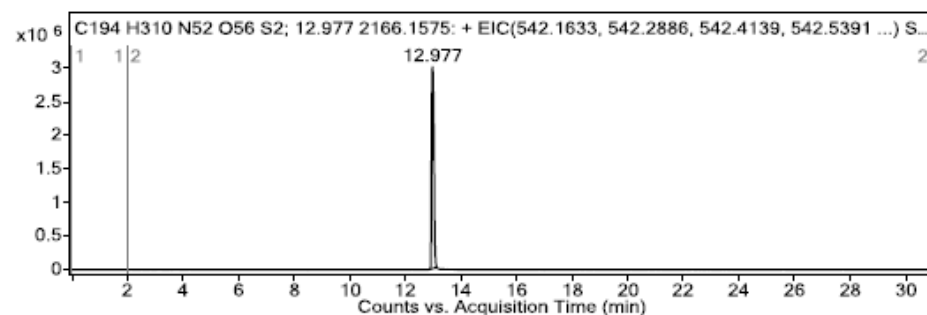
HPLC Chromatogram of San43



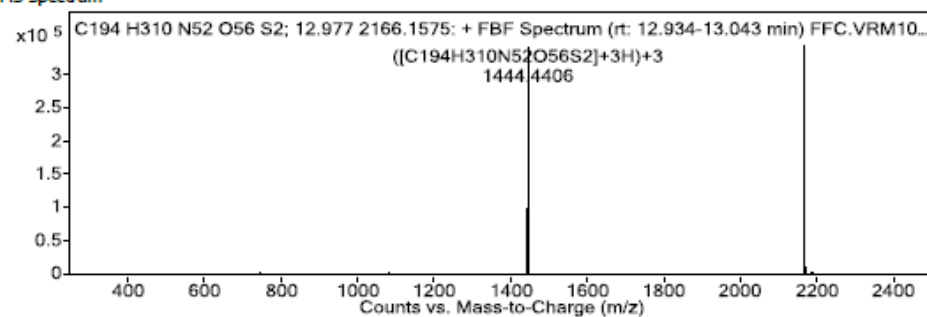
HPLC Chromatogram of San44



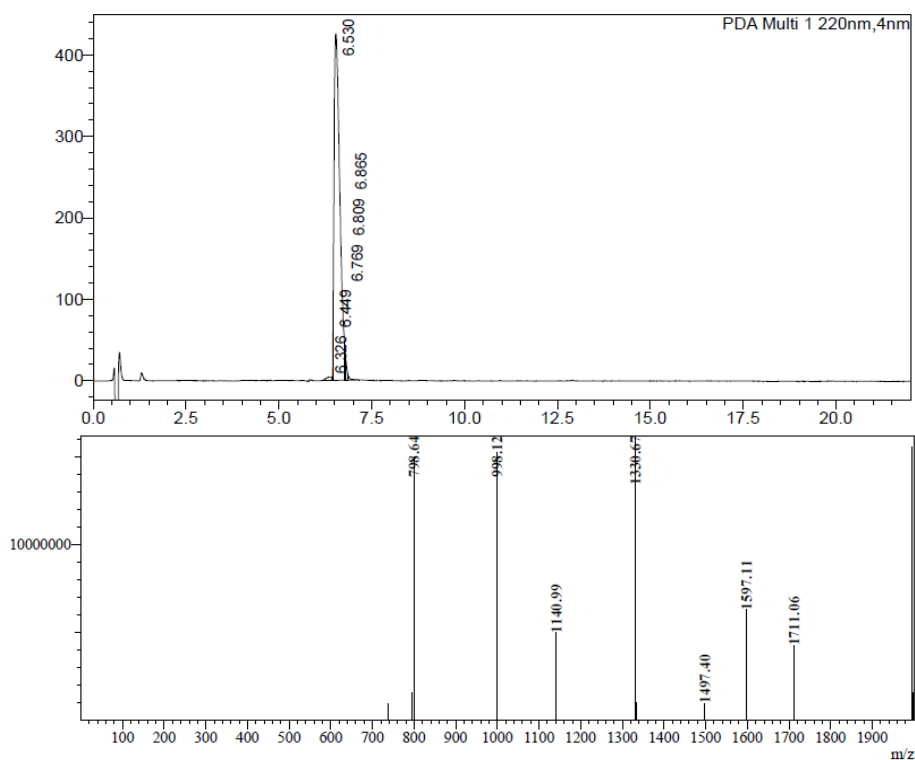
HPLC Chromatogram of San45



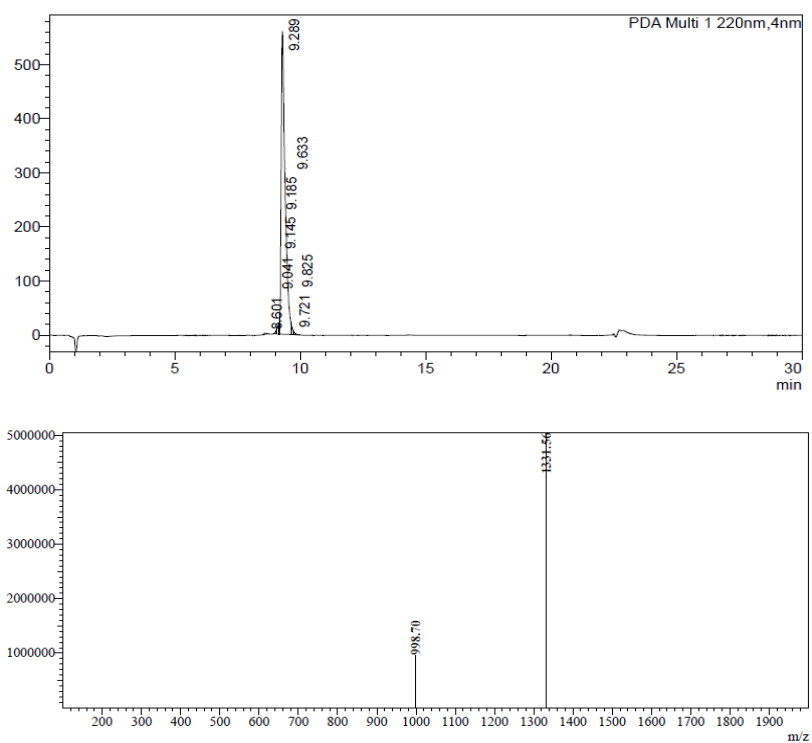
MS Spectrum



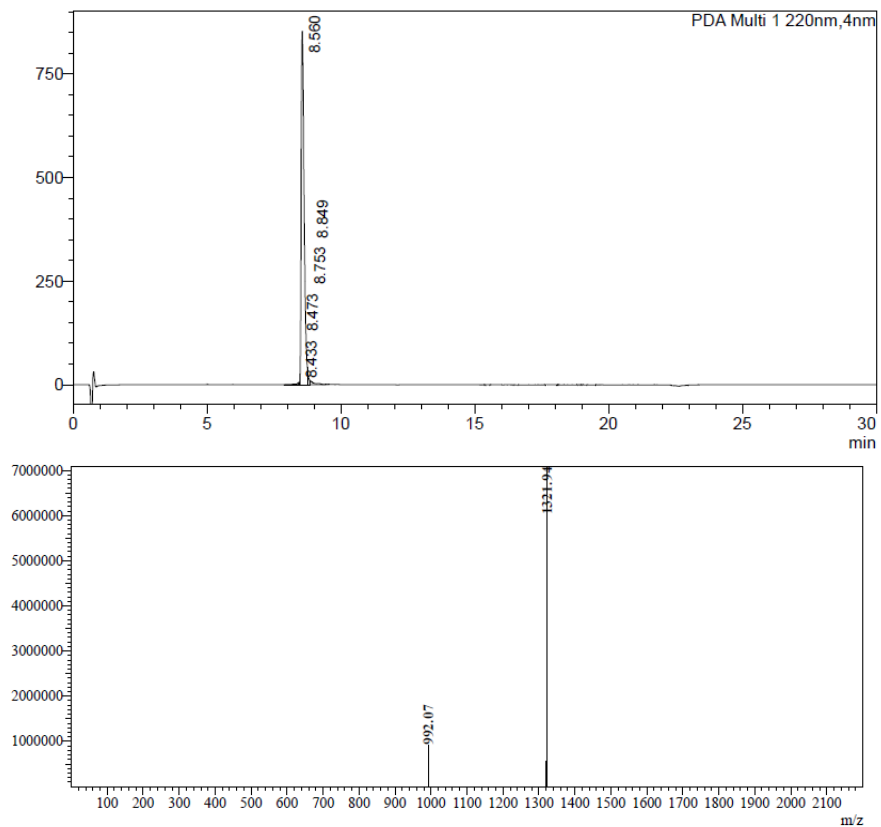
HPLC Chromatogram of San46



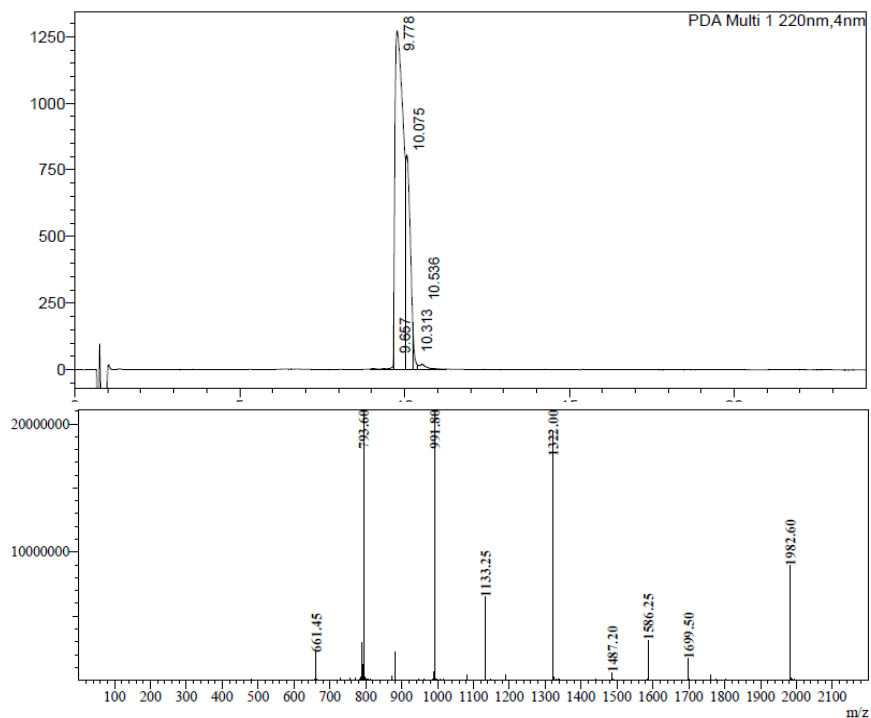
HPLC Chromatogram of San385



HPLC Chromatogram of San386



HPLC Chromatogram of San387



HPLC Chromatogram of San388

Supplementary reference

1. Wootten, D.; Simms, J.; Miller, L. J.; Christopoulos, A.; Sexton, P. M., Polar transmembrane interactions drive formation of ligand-specific and signal pathway-biased family B G protein-coupled receptor conformations. *Proc Natl Acad Sci USA* 2013, 110 (13), 5211-5216.